On page 19, ln. 30 – page 20, ln. 9, please replace the paragraph with the following:

Sixty grams of group A streptococcal cells in 600 ml water were combined with 75 ml of 4 N sodium nitrite and 75 ml of glacial acetic acid. The solution was mixed for 15 minutes and centrifuged for 10 minutes at 11,000 rpm in a SS34 rotor. The supernatant was removed, dialyzed against water and lyophilized. The group A polysaccharide was purified from the crude lyophilized extract by gel filtration through a SEPHADEX G-50 column (Pharmacia) using PBS as eluant. Fractions eluting from the column were monitored for the presence of carbohydrate using the phenolsulfuric acid assay of Dubois (31). The carbohydrate positive fractions were pooled, dialyzed at 4°C against water and lyophilized. The polysaccharide preparation (240 mg) contained less than 1% (w/w) proteins and nucleic acids. Its purity was further confirmed by ^{1}H-NMR at 500 MHz using an AM-500 BRUKER spectrometer.

Please replace the paragraph on page 20, ln. 24 – page 21, ln. 11 with the following:

ELISA Assays: The ELISA method was essentially that described by Fillit et al (18) with the following modifications. Preliminary testing with human sera indicated that 0.5 μg CHO/ ml in PBS, pH 7.2 of the liposomal preparation to sensitize the microtiter plates give the best results with minimal background readings against the liposomal control preparations. Accordingly, 100 μl of the preparation is placed per well in microtiter plates (Dynatech plates, USA) and incubated at 37°C overnight. The plates were then washed 3x in ELISA wash buffer (10 mM NaAcetate, 100 mM NaCl, 0.1% BRIJ 35, pH 8.0). The human sera was diluted in the same ELISA buffer and 100μl of a given serum dilution was placed in the plates and incubated 1 hour at 37°C. All sera were run in duplicate. After appropriate washes, 1:1,000 dilution of Goat F(ab')2 anti-human IgG (gamma chain specific) or IgM (Mu chain specific), alkaline phosphatase conjugate (Tago, Inc.,USA) was used as the secondary antibody and incubated for an additional hour at 37°C. After 3 additional washes in ELISA buffer, a phosphatase substrate (Sigma 104) in 0.1 M Diethanolamine, pH 9.6 was added to the wells, the plates incubated at 37°C for 1 hour and read on ELIDA V (Physica Co.) instrument at 405 nm. The titer was reported as that dilution which gave a reading of 1.0.

On page 22, lns. 16-34, please replace the paragraph with the following:

Absorption of N-acetylglucosamine antibodies from human sera: 600 μl of a 50% suspension of a N-acetylglucosamine coupled to SEPHAROSE beads (Sigma Chemical Co.) in PBS was placed into a sterile EPPENDORF tube and centrifuged at 4°C at 14,000 RPM for 10 minutes. The supernatant was removed and 300 μl of serum added to the beads. The suspension was rotated end over end for 1 hour at 37°C. Following a second centrifugation under the same conditions, the absorbed serum was removed and used in the bactericidal assay as described previously. To remove the N-acetylglucosamine antibodies from the affinity column, the beads containing the absorbed antibodies were packed in a 1 ml tuberculin syringe over which a solution of 0.58% (v/v) glacial acetic acid in 0.15 M NaCl, pH 2.2 is passed. The eluant is monitored by absorption at 280 nm and the peak fractions collected, dialyzed against PBS, pH 7.2, and concentrated back to the original volume of serum using an Amicon CENTRIPREP 30 concentrator (Amicon, Beverly, MA).

Please replace the paragraph on page 24, lns. 1-17 with the following:

Bactericidal Assays: Having established that human sera do contain group A carbohydrate antibodies and that the titers of these antibodies do vary in individuals, we next addressed the question of whether these antibodies would also promote opsonophagocytosis in an in vitro assay system. The bactericidal assay was essentially that used by Dr. Lancefield (15,25,26) for testing human sera with the modifications as outlined above. Figure 4 is illustrative of the results of the phagocytic assays. Using an inoculum of nine colony forming units of a serotype 6 group A Streptococcal strain, there was a marked increase in the number of colonies in the rotated tubes in the presence of normal rabbit serum (Figure 4A). Figure 4B shows a slight increase in the stationary tube in which the human serum was used. In marked contrast, the rotated tube containing the human serum (Figure 4C) completely abolished the growth of the organisms (compare Figures 4B and 4C).

On page 24, ln. 30 – page 25, ln. 6, please replace the paragraph with the following:

Relationship between the Anti-CHO Titers and opsonophagocytosis by human sera: Employing the phagocytic assay, it is clear that human sera differed in their ability to promote phagocytosis of group A Streptococci. In general the phagocytic properties of a given serum correlated with the titers of the antigroup A carbohydrate antibodies. As seen in Figure 7, all sera exhibiting titers greater than 200,000 exhibited greater than 80% killing, while three out of the four sera with titers less than 200,000 did not. One serum with a CHO titer of 40,000 did promote phagocytosis but the degree of killing was far less than that observed with high titered anti-CHO sera.

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Please replace the paragraph on page 25, ln. 31 – page 26, ln. 18 with the following:

Absorption Experiments: In an effort to determine which part of the streptococcal carbohydrate moiety was responsible for the bactericidal activity, human sera were absorbed with N-acetylglucosamine coupled SEPHAROSE beads as described in the methods section. Absorbed and non-absorbed sera were then used in the standard bactericidal assay. Figure 7 shows the results of these experiments. The unabsorbed serum clearly enhanced phagocytosis of the streptococci. In contrast, the serum absorbed with the N-acetylglucosamine coupled beads removed the opsonizing antibodies. As a viability control, normal rabbit serum did not enhance phagocytosis. These experiments indicate that the antibodies directed against the non-reducing terminal N-acetylglucosamine residue on group A carbohydrate were extremely important in the opsonophagocytosis of group A Streptococci in our bactericidal assays. To confirm these results, the antibodies from selected sera which had been absorbed to the N-acetylglucosamine affinity column were eluted and used in the bactericidal assay. As also shown in Figure 9, these experiments demonstrated that N-acetylglucosamine specific antibodies eluted from the affinity column were capable of partially restoring the opsonophagocytic bactericidal activity of the serum.

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REMARKS

Applicants respectfully request favorable reconsideration in view of the herewith presented amendments and remarks.